Journal of Power Sources 196 (2011) 9467–9472

Contents lists available at ScienceDirect

Journal of Power Sources

journal homepage: www.elsevier.com/locate/jpowsour

Electrochemically assisted methane production in a biofilm reactor

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ARTICLE INFO

Article history:
Received 21 April 2011
Received in revised form 24 June 2011
Accepted 5 July 2011
Available online 12 July 2011

Keywords:
Microbial electrolysis cell
Extracellular electron transfer
Methane production
Microbial biocathode
Activation energy

ABSTRACT

Microbial electrolysis is a new technology for the production of value-added products, such as gaseous biofuels, from waste organic substrates. This study describes the performance of a methane-producing microbial electrolysis cell (MEC) operated at ambient temperature with a *Geobacter sulfurreducens* microbial bioanode and a methanogenic microbial biocathode. The cell was initially operated at a controlled cathode potential of −850 mV (vs. standard hydrogen electrode, SHE) in order to develop a methanogenic biofilm capable of reducing carbon dioxide to methane gas using abiotically produced hydrogen gas or directly the polarized electrode as electron donors. Subsequently, *G. sulfurreducens* was inoculated at the anode and the MEC was operated at a controlled anode potential of +500 mV, with acetate serving as electron donor. The rate of methane production at the cathode was found to be primarily limited by the acetate oxidation kinetics and in turn by *G. sulfurreducens* concentration at the anode of the MEC. Temperature had also a main impact on acetate oxidation kinetics, with an apparent activation energy of 58.1 kJ mol^{-1}.

1. Introduction

Microbial electrolysis has recently emerged as a novel process for the renewable and sustainable production of biofuels or valuable chemicals from waste organic materials [1–4]. A microbial electrolysis cell (MEC) is a bioelectrochemical system (BES) consisting of an anode and a cathode, typically separated by an ion exchange membrane. At the anode, "electro-active" microorganisms oxidize organic or inorganic waste substrates using the electrode as terminal electron acceptor [5]. Electrons travel from the anode to the cathode where, in the presence of a suitable catalyst, reduce oxidized species into value-added products. Typically, MECs require the potential generated from substrate oxidation at the anode to be boosted with an external power supply in order to overcome the thermodynamic barrier and/or to drive the cathodic reaction at high rates. So far, the most studied cathodic reaction in MECs is hydrogen evolution (from protons reduction); this reaction typically requires the addition of an external voltage higher than 0.2 V and the presence of precious metal catalysts such as platinum [6,7]. In spite of these catalysts having excellent electrocatalytic activity towards hydrogen production, their high costs drastically limit their potential for practical application. Alternative low-cost cathodic catalysts, including carbon felt, stainless steel, tungsten carbide, and nickel alloys are currently being investigated, even though these materials often exhibit insufficient chemical stability and/or reactivity at neutral pH [8–12].

Recently, microbial biocathodes in which microorganisms are the electrocatalytic agents of the desired cathodic reaction have received considerable attention [13–17]. Main advantages of microbial biocathodes include self-regeneration of the catalyst, low cost, and greatest activity at neutral pH. Moreover, due to their high versatility and specificity, microbial biocathodes can potentially be employed to catalyze a wide spectrum of useful reactions. As an example, it has been recently demonstrated that microbial biocathodes can be employed to reduce carbon dioxide to methane gas via a process known as "electromethanogenesis" [18] or even multicarbon organic compounds via microbial "electrosynthesis" [2].

The MEC approach for methane production is particularly interesting, since in principle it can have several advantages compared to traditional anaerobic digestion processes, such as: (i) the physical separation of the organic matter oxidation from methane generation allows producing a biogas that is richer in methane; (ii) the methanogenic consortia are more protected against inhibitory compounds possibly present in the waste material; (iii) less thermal energy (if any) is needed to control the temperature of the cathode since the wastewater does not need to be warmed up, being processed only at the anode side; (iv) diluted streams can be used, including municipal wastewaters.

Anaerobic digestion and methane-producing MEC could also be operated in series, because of the possibility to remove the residual organics contained in the effluent of a conventional anaerobic digester with such a bioelectrochemical system, which is typically

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doi:10.1016/j.jpowsour.2011.07.016

effective even at low substrate concentrations and can be operated at ambient temperature [3].

So far, very few studies have investigated the application of MEC technology for methane production [3,18–21], therefore fundamental information regarding the startup of the system as well as the optimal anode and cathode potentials is still lacking. In this context, a recent literature review [22] has pointed out that controlling the BES anode at a high potential (i.e., >+0.3 V vs. standard hydrogen electrode, SHE) in most cases allows achieving a faster start-up time, higher current generation, and biomass production, and in turn better performance. The rationale behind this finding is that, in principle, high anode potentials allow microorganisms to gain more energy from substrate oxidation (provided they possess respiratory enzymes capable to function near to that potential and therefore to capture the available energy). High anode potentials can also enhance the kinetics of substrate oxidation by providing a high driving force for microbe-to-electrode extracellular electron transfer. In principle, similar considerations hold for the cathodic reaction where low potentials are expected to yield better performances [20]. As a main counterpart, the larger the difference between anode and cathode potentials, the higher the external energy input to the MEC. This clearly leads to a trade off between the potentials that are optimal for an energetically sustainable MEC operation and the potentials that are optimal for the microorganisms.

This study examined the performance of a methane-producing MEC, operated at ambient temperature, with a microbial (*Geobacter sulfurreducens*) bioanode and a microbial methanogenic biocathode. The MEC was started up by sequentially controlling the cathode and anode potentials at values that are favorable to the establishment of an active methanogenic biocathode and acetate-oxidizing bioanode, respectively. Successive inoculations of the cathode and anode compartments were also employed as a strategy to enhance reaction kinetics. Finally, the effect of ambient temperature fluctuations on process performance was also analyzed.

2. Materials and methods

2.1. Reactor set-up

The MEC employed in this study consisted of two identical Plexiglas frames, with internal dimensions of 17 cm × 17 cm × 3 cm, bolted together between two Plexiglas plates. A Nafion® 117 proton exchange membrane (PEM) was placed between the frames (Fig. 1A).

Prior to being used, the PEM was pretreated by boiling successively in H_2O_2 (3%, v/v), distilled water, 0.5 M H_2SO_4, and finally in distilled water again, for 2 h each. The total empty volume of each frame (i.e., of the anodic and cathodic compartments) was 0.86 L. The anodic and cathodic compartments were filled with graphite granules with a diameter between 2 and 6 mm (El Carb 100, Graphite Sales, Inc, USA) (Fig. 1B), giving a bed porosity of 48%. The specific electrodic surface area of each compartment was about 1290 $m^2\ m^{-3}$. Prior to using, the graphite granules were submerged for 24 h in 37% HCl and then for 24 h in a NaOH (1 M) solution. The washing process was repeated three times and then the granules were thoroughly washed with distilled water and dried at 100 °C. The purpose of this treatment was to remove metals and eliminate any potential organic residues from the graphite material [23]. External electrical connections were guaranteed by inserting graphite rod current collectors (5 mm diameter, Sigma–Aldrich, Italy) in each compartment. An Ag/AgCl reference electrode (+0.199 V vs. standard hydrogen electrode, SHE) (Amel s.r.l., Milan, Italy) was also placed in each compartment in order to measure and control the potential of each electrode. All voltages are reported with respect to SHE.

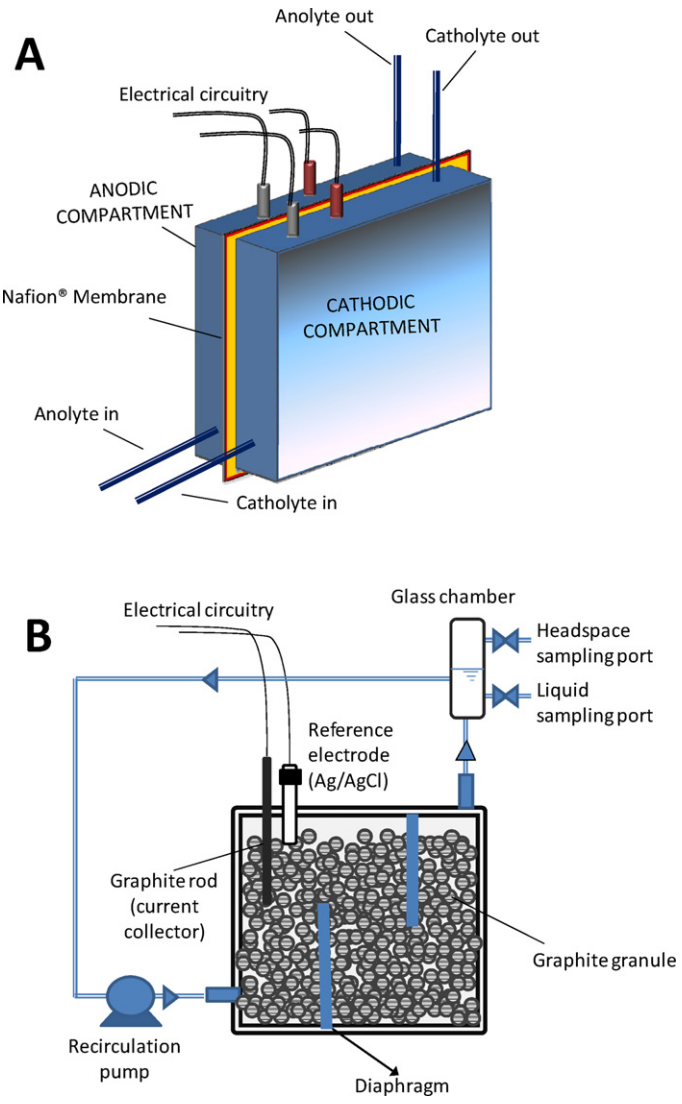

Fig. 1. Schematic overview of the methane-producing microbial electrolysis cell (MEC) (A). Schematic overview of a single MEC compartment (B).

The liquid phase in each compartment, hereafter referred to as anolyte and catholyte, was anaerobic basal medium which contained ($g\ L^{-1}$): NH_4Cl, 0.5; $MgCl_2{\cdot}6H_2O$, 0.1; K_2HPO_4, 0.4; $CaCl_2{\cdot}2H_2O$, 0.05; 10 $mL\ L^{-1}$ of a trace metal solution [24], and 10 $mL\ L^{-1}$ of vitamin solution [25]. The pH of the medium was maintained at values between 7 and 7.5 with a $NaHCO_3$ solution (10%, w/v).

The catholyte and the anolyte were continuously recirculated at a rate of 60 mL min^{-1}, using a peristaltic pump and Tygon® tubings, in order to prevent the establishment of substrate/product concentration gradients. The produced gas was collected in a glass chamber, equipped with sampling ports sealed with butyl rubber stoppers and aluminum crimps, which was inserted in each recirculation line. This chamber allowed sampling the headspace and the liquid phase of the MEC as well as spiking the anodic compartment with the substrate solution (Fig. 1B). The MEC was operated at room temperature (ranging from 21 to 25 °C), which was on-line monitored with an infrared thermometer (Testo SpA, Milan, Italy).

2.2. Reactor operation

Throughout this study the MEC was operated in a batch mode, with continuous recirculation of both the anolyte and the catholyte. During a first period of operation of approximately 20 days, referred to as Run 1, the cathode compartment was inoculated with 100 mL of a mixed hydrogenophilic methanogenic culture, previously enriched in a fill and draw mode on hydrogen and carbon dioxide as electron donor and acceptor, respectively [19]. The average cell concentration of the methanogenic culture, as volatile suspended solids (VSS), was around 80 mg L^{-1}. A detailed kinetic and molecular characterization of the methanogenic culture was reported elsewhere [19]. Two additional inocula of the same amount were carried out at different times during Run 1. Throughout this operation period, the cathode potential was controlled at −850 mV with a potentiostat (Bio-Logic, Grenoble, France), which also allowed measuring and recording the electrical current flowing in the system.

Prior to inoculating the cathode compartment with the methanogenic culture, abiotic experiments were carried out under the same conditions as in Run 1 (i.e., cathode potentiostatically controlled at −850 mV; abiotic anode).

During a successive period of operation, lasting around 45 days and referred to as Run 2, the anode compartment was inoculated with 100 mL of a *G. sulfurreducens* culture. To establish this culture, 1 mL of actively growing *G. sulfurreducens* cells (DSMZ culture collection, Braunschweig, Germany) was added to a serum bottle incubated at 30 °C and containing 100 mL of the anaerobic basal medium supplemented with acetate (to a final concentration of 10 mM) as electron donor and fumarate (40 mM) as electron acceptor. The *G. sulfurreducens* culture was transferred into the anode compartment only after most of the substrates were consumed in the serum bottle and the cell concentration had reached a target value of about 200 mgVSS L^{-1}.

During Run 2, the anode potential was controlled at +500 mV with the potentiostat, whereas the potential of the cathode was periodically monitored with a digital multimeter (Keithley Instruments, Cleveland, OH). Acetate was supplied to the anode (to a final concentration between 10 and 15 mM) by spiking the glass chamber in the recirculation line with a concentrated sodium acetate solution (1 M). The addition of acetate (10 mM) caused an increase of the conductivity of the medium from 4.80 mS cm^{-1} to 6.50 mS cm^{-1}, whereas it did not significantly affect the pH. Preliminary experiments had indicated that acetate was not abiotically oxidized at +500 mV.

At different times both the anode and the cathode were repeatedly inoculated with *G. sulfurreducens* and the methanogenic culture, respectively. Each inoculum of *G. sulfurreducens* roughly corresponded to 20 mg of biomass as volatile suspended solids (VSS), whereas each inoculum of methanogenic culture corresponded to about 8 mg of VSS.

In both Run 1 and Run 2, about 15% (v/v) of the catholyte and the anolyte were removed weekly from the reactor and replaced with fresh anaerobic basal medium. The pH was adjusted by using a $NaHCO_3$ (10%, w/v) or an HCl (1 M) solution.

2.3. Analytical measurements and calculations

The concentration of microorganisms in the cultures used to inoculate the MEC was determined as VSS, according to standard methods [26]. Acetate was analyzed by injecting 1 μL of filtered (0.22 μm porosity) aqueous sample into a Dani Master gas-chromatograph (2 m × 2 mm glass column packed with Carbopack, He carrier gas 25 mL min^{-1}; oven temperature 175 °C; flame ionization detector (FID) temperature 200 °C). Methane was analyzed by injecting 50 μL of sample headspace (with a gas-tight Hamilton syringe) into a Varian (Lake Forest, CA, USA) 3400 gas-chromatograph (GC; 2 m × 2 mm glass column packed with 60/80 mesh Carbopack B/1% SP-1000; He carrier gas at 18 mL min^{-1}; oven temperature at 50 °C; FID temperature 260 °C). Hydrogen was analyzed in a 500 μL gaseous sample by a Trace Analytical (Menlo Park, CA, USA) TA3000R gas-chromatograph (molecular sieve packed column, N_2 carrier gas 24 mL min^{-1}; oven temperature 105 °C, reduction gas detector (RGD) temperature 285 °C) (H_2 detection limit 0.02 ppmv). When the hydrogen level was above the range of the RGD (i.e., about 100 ppmv), it was quantified using a Varian 3400 gas-chromatograph (stainless-steel column packed with molecular sieve; He carrier gas 18 mL min^{-1}; oven temperature 180 °C; thermal-conductivity detector (TCD) temperature 200 °C) [27]. Headspace concentrations were converted to aqueous-phase concentrations using tabulated Henry's law constants [28].

The cumulative electric charge (meq_i) that was transferred at the electrodes was calculated by integrating the current (A) over time and dividing for the Faraday's constant (F = 96 485 C eq^{-1}). Cumulative equivalents recovered as methane (meq_{CH_4}) or hydrogen (meq_{H_2}) were calculated from their measured amounts (mmol), considering the molar conversion factors of 8 meq $mmol^{-1}$ and 2 meq $mmol^{-1}$ for methane and hydrogen, respectively. Cumulative equivalents released from the oxidation of acetate (meq_{AC}) were calculated from the measured amount (mmol) of acetate consumed, considering the corresponding molar conversion factor of 8 meq $mmol^{-1}$. The rates of methane and hydrogen production were calculated from the slope of the curve reporting the meq as a function of time and normalized to the total empty volume of the cathode (0.86 L). All rates were normalized to 24.3 °C (i.e., the average ambient temperature during the period of operation) using experimentally determined activation energy value.

The cathode capture efficiency (CCE) represents methane recovery from current and was calculated as $CCE_{CH_4}(\%) = (meq_{CH_4}/meq_i) \times 100$. The coulombic efficiency represents current generation from acetate and was calculated as $CE_{AC}(\%) = (meq_i/meq_{AC}) \times 100$.

The energy efficiency relative to electrical input was calculated as $\eta_E(\%) = W_{CH_4}/W_{IN}$, where $W_{CH_4} = n_{CH_4} \times \Delta G_{CH_4}$ is the energy recovered (kJ) as methane, calculated from the total amount of CH_4 produced (n_{CH_4}, mol) and the molar Gibbs free energy of CH_4 oxidation by oxygen to carbon dioxide ($\Delta G_{CH_4} = -817.97$ kJ mol^{-1}) [29], W_{IN} (kJ) is the electrical energy added to the system, calculated as $C \times E_{APP}$, where C is the total Coulombs calculated by integrating the current over time and E_{APP} (V) is the applied voltage calculated as the difference between the cathode and anode potentials. The energy efficiency relative to substrate input was determined as $\eta_S(\%) = W_{CH_4}/W_S$, where $W_S = n_S \times \Delta G_S$ is the energy content of the substrate added, calculated from the amount of acetate consumed (n_S, mol) and the molar Gibbs free energy of acetate oxidation to carbon dioxide (−844.61 kJ mol^{-1}) [29]. The overall energy recovery: $\eta_{E+S}(\%) = W_{CH_4}/(W_{IN} + W_S)$, took into account both electric and substrate energy inputs.

2.4. Chemicals

Methane, hydrogen (99.5+%) and all the other chemicals were purchased from Sigma–Aldrich (Milan, Italy) (except where differently indicated). The other chemicals used to prepare the mineral medium were of analytical grade and were used as received.

3. Results and discussion

3.1. Start-up and operation of the methane producing biocathode (Run 1)

In order to startup the methane-producing biocathode, the MEC was operated at a cathode potential of −850 mV. Potentiostatic

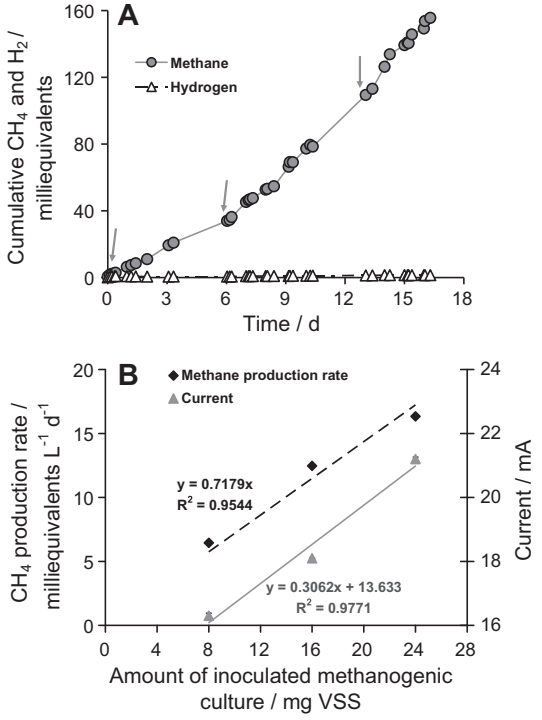

Fig. 2. Time course of cumulative methane and hydrogen production throughout Run 1 with the cathode potential controlled at −850 mV (vs. SHE); arrows indicate inoculations of the cathode with the methanogenic culture (A). Effect of the amount of methanogenic biomass at the cathode on the rate of methane production and average electric current (B).

experiments carried out at this potential prior to inoculating the cathode, had indicated the occurrence of abiotic hydrogen production at a rate of 6.3 meq L^{-1} d^{-1}, which was expected to support the establishment of an active methanogenic biofilm.

Fig. 2A shows the cumulative amount of methane and hydrogen produced during Run 1 (both the amount at the cathode and at the anode were accounted). Upon a first inoculation (on day 0) with approximately 8 mg VSS of biomass, methane production started immediately, without any initial lag phase, at a rate of 6.4 meq L^{-1} d^{-1} (hence very close to the rate of abiotic hydrogen production rate). Throughout the run, hydrogen concentration remained relatively low, most likely due to its rapid consumption (via hydrogenophilic methanogenesis) by the microbial culture. Apparently, the PEM was highly permeable to methane and hydrogen gases which were constantly present at the cathode and anode compartments in comparable amount, as previously reported [30]. Notably, the rate of methane production almost linearly increased with a second (on day 6) and a third (on day 13) inoculation of the methanogenic culture at the cathode (Fig. 2B).

This clearly indicated that the performance of the biocathode was primarily limited by the concentration of microorganisms in the cathodic compartment. Accordingly, also the cathodic current linearly increased, as reported in Fig. 2B.

Interestingly, the average current measured at −850 mV in the abiotic control experiments was 16.8 ± 0.6 mA, hence very close to the value measured after the first inoculation (16.3 mA) but substantially lower than values measured after the second (18.1 mA) and third (21.2 mA) additions of methanogens. The observed increase of both the cathodic current (up to 30% in correspondence to the third inoculation) and methane production rate (up to 2.5 times) could be due to the gradual onset of "electromethanogenesis" [18,19], whereby microorganisms directly accept electrons from the surface of the poalrized cathode. Another possible explanation is that microorganisms indirectly increased the current by

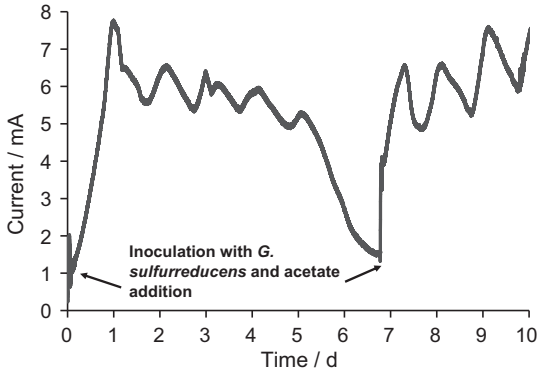

Fig. 3. Current generation by *G. sulfurreducens* during the initial ten days of operation of Run 2, with the anode potential poised at +500 mV (vs. SHE).

enhancing the removal of hydrogen (i.e., the product of abiotic proton reduction) from the electrode surface. Further investigation is needed to address this issue.

Cathode capture efficiency, relative to methane formation, steadily increased during Run 1 from 38% to 74%. The accumulation of gas bubbles (most likely consisting of methane gas) within the granular graphite bed could have resulted in an underestimation of the produced methane and could have caused the incomplete methane recovery. Another possible explanation could have been the oxidation at the anode of the hydrogen produced at the cathode, as previously reported [21].

3.2. Performance of the MEC at a controlled bioanode potential of +500 mV (Run 2)

At the start of Run 2, the anode was inoculated with 20 mg VSS of *G. sulfurreducens*, spiked with acetate (serving as electron donor) to a concentration of around 15 mM, and then polarized to an oxidizing potential of +500 mV. Electric current began to increase almost exponentially, soon after inoculation and reached a peak value of ~8 mA after approximately 1 day (Fig. 3). Thereafter, the current remained in the range between 5.5 and 6.5 mA until day 5, when it rapidly dropped below 2 mA due to the exhaustion of the acetate (data not shown). The current, however, rapidly resumed following a new acetate addition and a further inoculation of *G. sulfurreducens*. Throughout the remainder of Run 2, acetate was re-spiked whenever it was below 5 mM.

The current measured in the presence of excess acetate exhibited significant cyclic fluctuations which were closely correlated to the daily variations of ambient temperature. As an example, Fig. 4A shows the existing match between electric current and temperature, relative to the period of MEC operation from day 26 to day 38.

As current is proportional to the rate of acetate oxidation reaction, which is in turn proportional to the rate constant, experimental data (collected throughout most of Run 2, under non-limiting substrate concentration) were plotted in Arrhenius form ($\ln i$ vs. $1/T$) to determine the "apparent" activation energy of the reaction (i.e., the current response of the system to temperature variations) (Fig. 4B). The obtained value of 58.1 ± 2.4 kJ mol^{-1} is comparable to that recently reported for the catalytic oxidation of acetate by a mixed-culture biofilm (i.e., 44.85 kJ mol^{-1}) [31].

It is also important to note that the value determined in this study falls within the range of those typically reported for (bio)chemical reactions (30–80 kJ mol^{-1}) [32], thereby suggesting that intrinsic kinetics of acetate oxidation with the electrode serving as electron acceptor, rather than mass-transport of the substrate or products, was rate-limiting current generation.

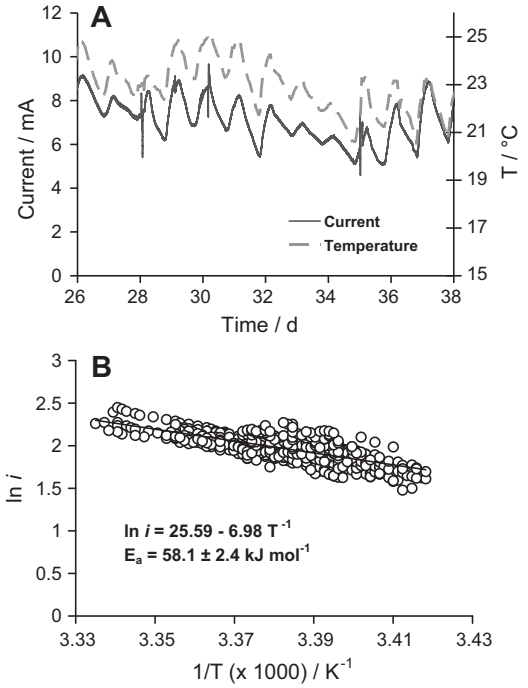

Fig. 4. Fluctuations of electric current and ambient temperature relative to the period of Run 2 from day 26 to day 38 (A). Dependence of electric current on temperature: Arrhenius plot (B).

Fig. 5 shows the cumulative acetate oxidized, electric charge transferred and methane produced throughout Run 2. Both the anode and the cathode were repeatedly inoculated with the *G. sulfurreducens* (around 20 mg as VSS, unless otherwise indicated) and the methanogenic culture (around 8 mg VSS) respectively, in order to verify the possibility to enhance reaction kinetics.

On an electron equivalent basis, the cumulative electric charge transferred to the anode typically exceeded the oxidized acetate and, as a consequence of that, the coulombic efficiency relative to acetate oxidation was above 100%. This finding was probably due to electron recycling through the oxidation on the anode of the hydrogen produced at the cathode and diffusing through the PEM, as also previously suggested in the literature [21].

During acetate oxidation with the anode polarized at +500 mV, the cathode potential was typically in the range between −650 and −700 mV, hence substantially higher (less-reducing) than in Run 1

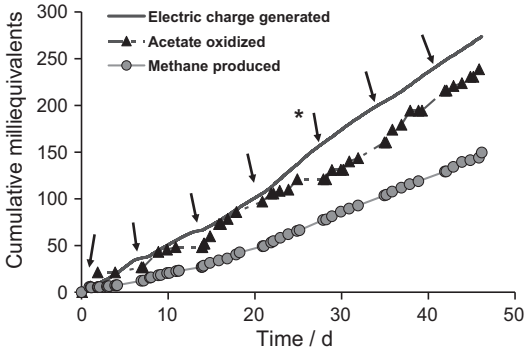

Fig. 5. Cumulative values of electric charge generated, acetate oxidized, and methane produced throughout Run 2, with the anode electrode poised at +500 mV (vs. SHE). Arrows indicate inoculation of about 20 mg (as VSS) of *G. sulfurreducens* culture and 8 mg of methanogenic culture at the anode and the cathode of the MEC, respectively. In correspondence to the asterisk, the anode was inoculated with a double amount of *G. sulfurreducens*.

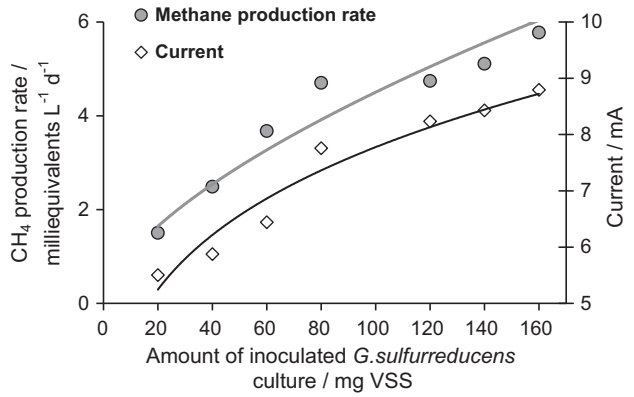

Fig. 6. Average value of electric current and methane production rate as a function of the amount of *G. sulfurreducens* added to the anode of the MEC.

(i.e., −850 mV). In spite of that, methane formation was the dominant electron accepting reaction, with a CCE that increased over time up to nearly 65% in correspondence to the last inoculations, a value that is close to that obtained in Run 1, when a cathodic polarization was applied. The apparent hydrogen production was very low, likely due to both its rapid consumption by methanogens and its diffusion into the anodic compartment, as previously discussed.

The average values of electric current and methane production rate, achieved after each inoculum, were calculated from the slope of the cumulative electric charge and methane produced. As shown in Fig. 6, both the electric current and methane production rate increased with the amount of *G. sulfurreducens* added to the anode.

In a potentiostatically controlled MEC, an increase of electric current denotes an increase in the kinetics of the reaction taking place at the electrode being controlled. Hence, the positive effect of *G. sulfurreducens* inocula on the electric current indicates that the concentration of these microorganisms was actually rate-limiting the performance of the system. The corresponding increase of methane production rate indicates that methanogens (already present and freshly inoculated into the cathode) could promptly respond to the increased current by increasing the rate of hydrogenophilic methanogenesis and/or "electromethanogenesis". Notably, current and methane production rate increased almost linearly with the first four inoculations; subsequently their rate of increase slightly diminished, suggesting that other factors, in addition to *G. sulfurreducens* concentration at the anode, were also limiting the MEC performance.

It is worth mentioning that the maximum rate of methane production obtained during Run 2 (with the anode potential set to +500 mV) was approximately 3 times lower than that obtained by controlling the cathode potential at −850 mV. This provides further indications that the process was rate-limited by the kinetics of acetate oxidation at the anode and in turn of current generation rather than by the kinetics of methane formation (via hydrogenophilic methanogenesis and/or electromethanogenesis). Clearly, the lower methane production rate has to be related to the higher value of the cathode potential (i.e., between −650 and −700 mV), the latter resulting from the current generated by *G. sulfurreducens*. It is, therefore, expected that methane production rate could possibly be enhanced by further increasing the concentration and activity of *G. sulfurreducens* at the anode of the MEC.

3.3. MEC performance and efficiency

The energy efficiency of the MEC potentiostatically controlled at +500 mV, relative to the electrical energy input (η_E), was 57% (in correspondence to the last inoculations). By taking into account the energy input also deriving from the acetate consumed, the

Table 1
Performance of hydrogen- and methane-producing MECs reported in the literature versus values obtained in this study.

MEC configuration	Substrate	Operating conditions	Main product	T (°C)	Production rate ($L L^{-1} d^{-1}$)	η_E (%)	η_{E+S} (%)	Reference
Two chambers	Acetate	E_{APP} = 0.5 V	H_2	30	0.020	169[a]	53[a]	[33]
Single chamber	Acetate	E_{APP} = 0.6 V	H_2	30	0.53	204[a]	58[a]	[34]
		E_{APP} = 0.4 V			0.20	267[a]	27[a]	
Single chamber	Acetate	E_{APP} = 0.9 V	H_2	30	0.35–1.50	46–107[a]	19–46[a]	[8]
Single chamber	Acetate	E_{APP} = 1.0 V	CH_4	30	N.R.	N.R.	80[a]	[18]
Single chamber	Acetate	E_{APP} ≈0.8 V	CH_4	22	0.17–0.75	240–84[b]	36–55[b]	[3]
Single chamber	Acetate	E_{APP} = 0.9 V	CH_4	30	0.12	67[b]	30[b]	[21]
Single chamber	Acetate	Anode poised at 0.0 V	CH_4	35	0.53	70[b]	41[b]	[20]
Two chambers	Acetate	Anode poised at +0.5 V	CH_4	24	0.018	57[b]	30[b]	This study

N.R., not reported.
[a] Calculations based on heat of combustion.
[b] Calculations based on Gibbs free energy.

energy efficiency (η_{E+S}) became 30%. The potentiostatic control of the anode potential at +500 mV resulted in a voltage difference between the anode and the cathode of around 1.2 V, a value that is larger than those commonly applied in hydrogen and methane producing MEC (i.e., 0.4–1.0 V). In spite of that, the energy efficiencies obtained in this study are yet comparable with those achieved in other studies (Table 1). This is largely due to the fact that controlling the anode at a highly oxidizing potential allowed achieving a very high coulombic efficiency (i.e., ≈100%) relative to acetate oxidation. Another main advantage resulting from the potentiostatic mode of operation is that the anode potential was not limiting the rate of acetate oxidation, which accordingly was found to be almost linearly dependent on the biomass concentration at the anode. In spite of that, however, the maximum rate of current generation and methane production obtained in the present study was lower than those typically reported in the literature. This could be due to the fact that the total biomass inoculated to the anode (<200 mg VSS of *G. sulfurreducens*) was lower than that adopted in other studies.

However, considering that the anode surface area was far from being fully saturated by microorganisms, it is expected that both current and methane production rates could be dramatically increased through additional inoculations.

4. Conclusions

A two chamber microbial electrolysis cell was developed that generated methane gas from acetate at ambient temperature by using a methanogenic microbial biocathode coupled to a *G. sulfurreducens* microbial bioanode. The cell performance, operated either under potentiostatic biocathode or bioanode control, was markedly limited by the concentration of microorganisms present in each compartment. The apparent activation energy of acetate oxidation, estimated when the cell was operated under potentiostatic control of the bioanode, was found to be 58.1 kJ mol^{-1}, hence fully in the range of values reported for biological reactions. This finding further supports the indication that intrinsic kinetics of acetate oxidation with the electrode serving as electron acceptor, rather than mass-transport of the substrate or products, was rate-limiting current generation.

Overall, the obtained results suggest the possibility to further optimize the process by saturating the electrodic surfaces with microorganisms.

Acknowledgement

This study was carried out in the frame of the FITOLISI research project, funded by the Italian Ministry of Agriculture, Food, and Forestry (MIPAAF).

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